Francesco Celandroni Università di Pisa

Comportamento di *Bacillus cereus* in prodotti lattiero-caseari ed espressione della virulenza

GIORNATA STUDIO SULLE PROBLEMATICHE ATTUALI DEL SETTORE LATTIERO-CASEARIO

tossinfezioni alimentari

Aspetti epidemiologici

In Italia

In Italia, la sorveglianza dei focolai di tossinfezione alimentare avviene secondo il flusso previsto dal DM del 15 dicembre del 1990. Il Decreto prevede la suddivisione delle malattie infettive in 5 classi. In particolare la quarta (su notifica effettuata dal medico entro 24 ore dal sospetto di un caso di malattia) include le infezioni, tossinfezioni e infestazioni di origine alimentare (quando si verificano in forma di focolaio). Tuttavia questi dati sono spesso distorti, oltre che dalla sottonotifica, dalla mancata diagnosi eziologica attribuibile a uno scarso ricorso ad accertamenti di laboratorio. Inoltre, la trasmissione delle informazioni è spesso poco tempestiva e non permette di condurre tutte le indagini necessarie a stabilire la fonte e le modalità di trasmissione. Su notifica del medico, le Aziende sanitarie trasmettono alla Regione la segnalazione dei patogeni responsabili, in classi diverse a seconda della pericolosità dell'agente e dell'estensione dell'episodio (caso singolo o focolaio epidemico).

Nel 2009, in Italia, il numero di segnalazioni di focolai di tossinfezioni alimentari è stato di 248. Per ogni focolaio segnalato, il totale di casi è stato pari a 1451. L'Emilia Romagna è risultata essere la regione che segnala il maggior numero di episodi (20% del totale nazionale), seguita da Piemonte (15%), Provincia autonoma di Bolzano (14%), Lazio (10%) e da tutte le altre Regioni.

I microrganismi maggiormente implicati nell'eziologia degli episodi sono rappresentati dalle Salmonelle spp. (45%), seguite da forme virali (17%). Il *Campylobacter* risulta essere implicato solo nel 1,2% dei casi al contrario di quanto osservato in altri Paesi europei. Purtroppo il 33% dei focolai epidemici non presenta indicazione sull'eziologia degli episodi o non specifica il microrganismo responsabile.

Bacillus cereus: spore forming foodborne pathogen associated with two types of gastrointestinal diseases

emetic syndrome (small ring-shaped peptide called cereulide, which is pre-formed in food before ingestion)

diarrheal syndrome (one or more enterotoxins produced by live bacteria in the small intestine)

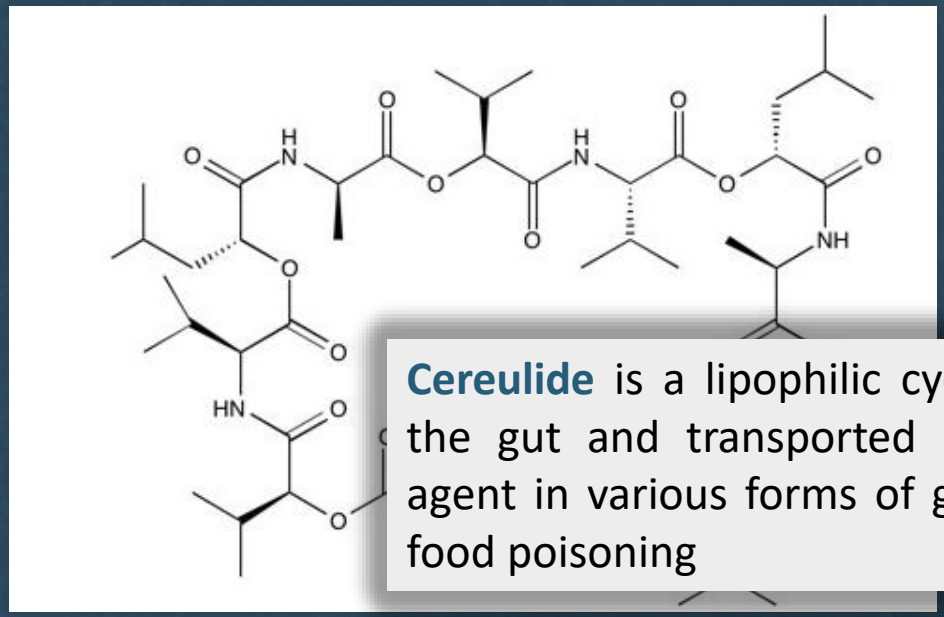
Disease related to the consumption of foods like vegetable purees (e.g. zucchini), soups, pasta and rice dishes and dairy products.

B. cereus is a common contaminant of raw milk but it is also frequently isolated from dairy products such as ice creams, milk powders, fermented milks, pasteurized milk and ricotta

Among the enterotoxins produced by *B. cereus*, the hemolytic enterotoxin hemolysin BL (**HBL**), the non-hemolytic enterotoxin (**Nhe**), and cytotoxin K (**CytK**) are claimed to play a major role in diarrheal disease

Tissue-destructive/reactive proteins damaging the integrity of the plasma membrane of several cells, epithelial cells of the small intestine included

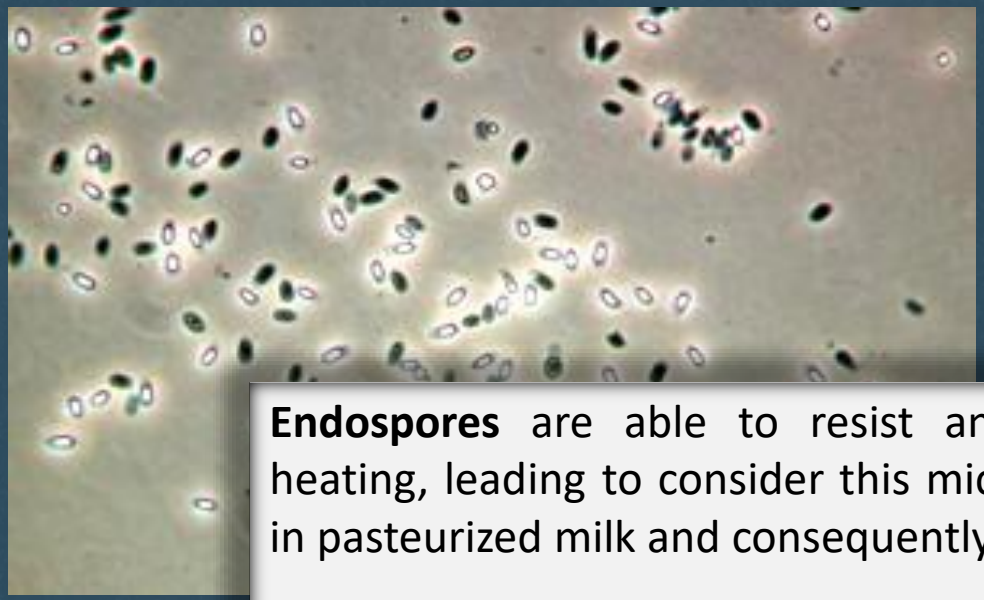
Denaturazione: 75°C 1-2 min



Cereulide is a lipophilic cyclopeptide and is rapidly absorbed in the gut and transported into the bloodstream. The causative agent in various forms of gastroenteritic disease associated with food poisoning

It is very hydrophobic, making it essentially insoluble in aqueous solution, which suggests that in food poisoning it is delivered to its target cells bound to, or dissolved in, carriers found in food. This propensity to bind to solid food or culture media components may lead to an underestimation of cereulide activity if particulates are removed by filtration or centrifugation

Highly thermoresistant (up to 90 min 130°C)



Endospores are able to resist and survive the pasteurization heating, leading to consider this microorganism a potential hazard in pasteurized milk and consequently in some dairy products

Highly hydrophobic: adhesion to various surfaces

Spore germination favoured by various substances (aminoacids) and thermal shock (60-80°C 10 min)

Vegetative cells growth usually occurs within the temperature range of 10-50°C – psychrotolerant strains

Centers for Disease Control and Prevention
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SEARCH

What types of outbreaks would you like to include? Select All

☑ Food ☐ Water ☐ Animal Contact ☐ Environmental ☐ Person to Person ☐ Indeterminate/Unknown

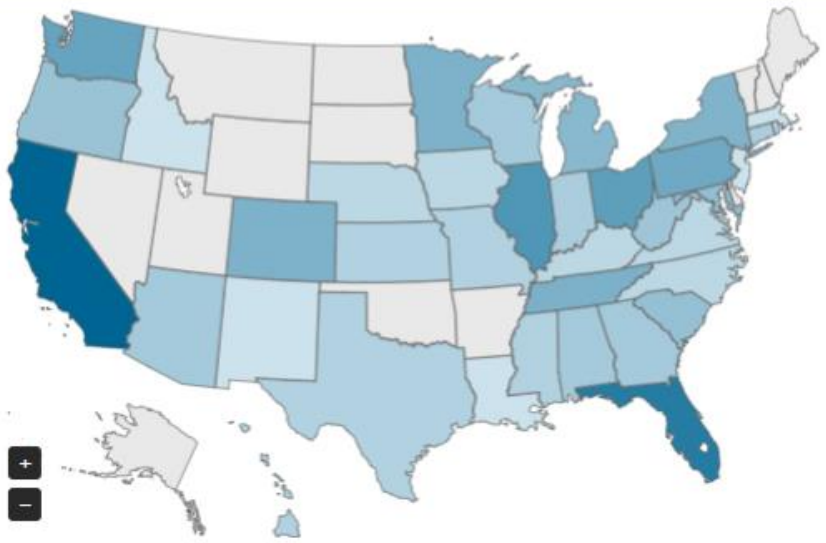
Current Search: 1998 to 2016 | Bacillus Clear

Filter Options:

- Year
- State
- Etiology
  - Select to Add
  - ☑ Bacillus
- Setting
- Food/Ingredient

Outbreaks per State

Display: U.S. Map



Quick Stats - Current Search

647	Outbreaks
8,293	Illnesses
76	Hospitalizations
3	Deaths

Quick Stats - Overall

41,269	Outbreaks
1,054,151	Illnesses
27,909	Hospitalizations
1,290	Deaths

Year Display: Deaths

Month Display: Outbreaks

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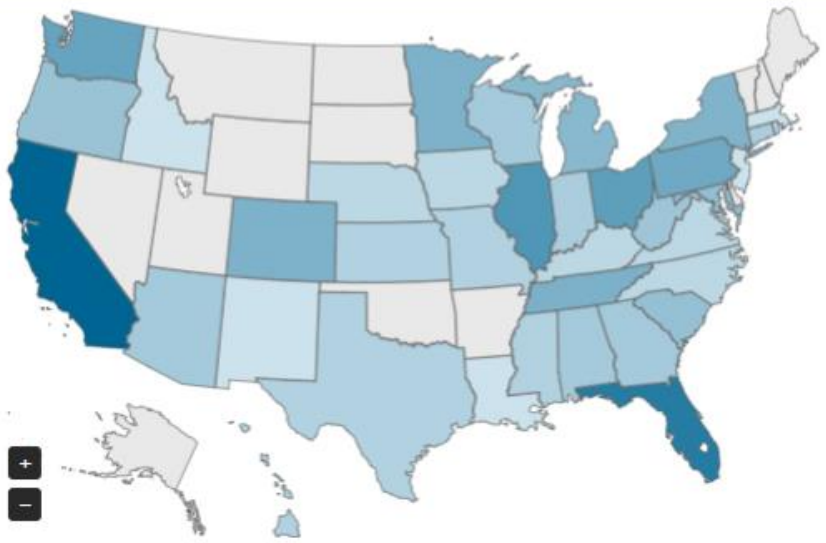

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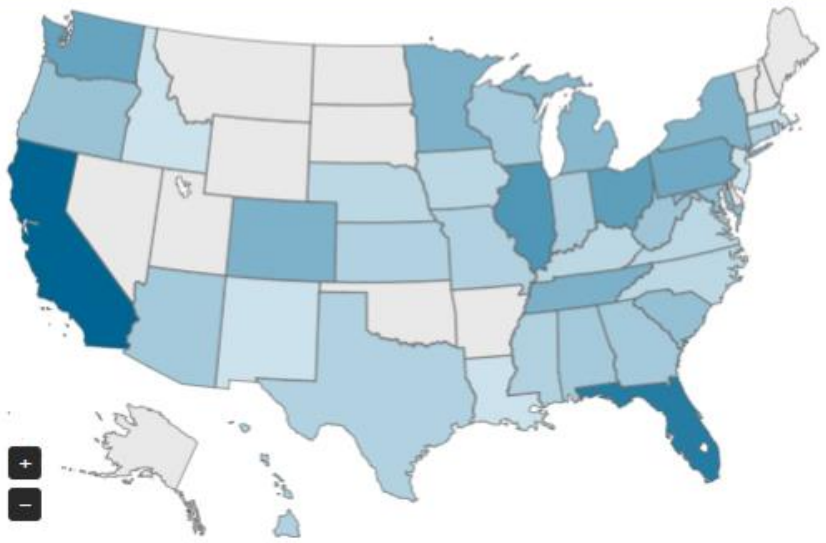

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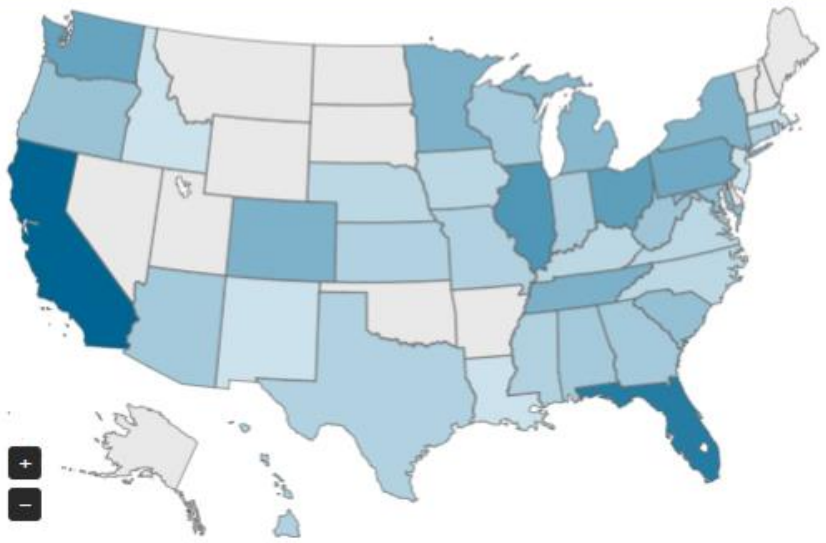

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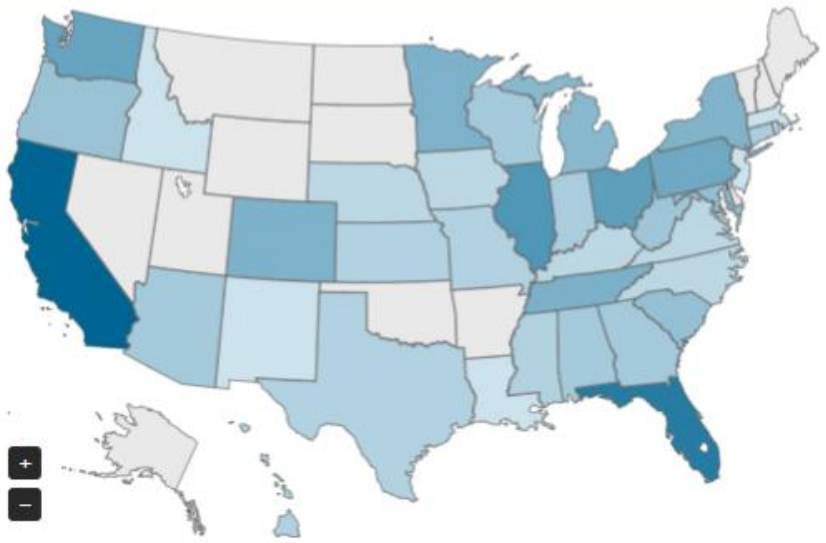

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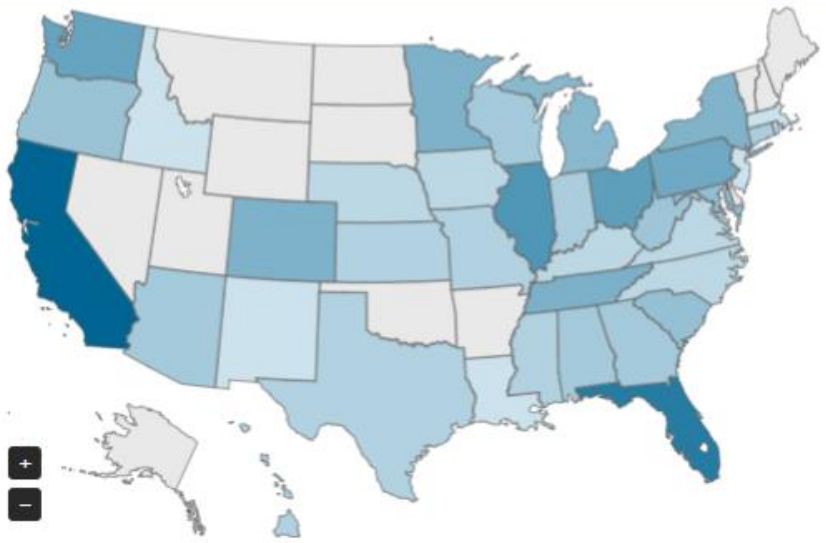

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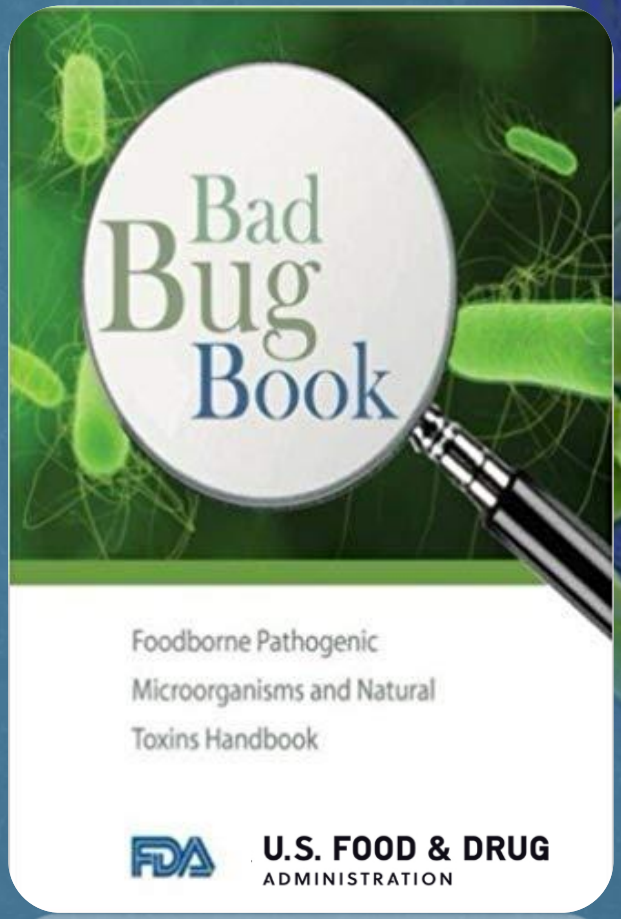

The **Bad Bug Book** estimated 63,400 episodes of *B. cereus* illness annually in the United States

Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs

efsa
European Food Safety Authority

SCIENTIFIC OPINION

Food vehicle	Year	Total number of food-borne outbreaks	Human cases	Hospitalisations
Bakery products	2008	2	25	2
	2010	1	8	0
	2011	4	29	0
	2012	1	5	0
Total – Bakery products		8	67	2
Poultry meat and products thereof	2007	7	95	1
	2008	1	10	0
	2009	6	63	8
	2011	3	47	0
	2012	3	46	0
	2014	2	5	0
Total – Poultry meat and products thereof		22	266	9
Red meat and products thereof	2007	8	40	0
	2008	4	175	3
	2009	5	59	0
	2011	4	98	0
	2012	5	256	1
	2013	6	145	0
	2014	2	27	0
Total – Red meat and products thereof		33	752	4
Mixed food or buffet meals	2007	12	83	0
	2008	18	307	32
	2009	17	401	12
	2010	10	349	3
	2011	10	94	16
	2012	13	216	5
	2013	19	306	81
	2014	15	319	32
Total – Mixed food or buffet meals		114	2,075	181
Cheese, milk or dairy products	2007	4	23	0
	2008	2	5	0
	2009	1	20	NR
	2010	1	2	0
	2011	1	3	3
	2012	1	2	0
	2013	1	10	0
Total – Cheese, milk and dairy products		11	65	3
Canned food products	2010	1	62	0
Total – Canned food products		1	62	0
Cereal products including rice and seeds/pulses (nuts, almonds)	2007	5	62	0
	2008	6	165	2
	2009	7	79	0
	2010	7	28	0
	2011	6	37	4
	2012	5	63	10
	2013	5	27	0
	2014	4	32	0
Total – Cereal products including rice and seeds/pulses (nuts, almonds)		45	493	16
Crustaceans, shellfish, molluscs and products thereof	2007	3	36	2
	2008	1	2	0
	2009	3	25	0
	2010	1	2	0
	2011	1	2	NR
	2013	1	2	0
	2014	2	8	0
Total – Crustaceans, shellfish, molluscs and products thereof		12	77	2
Fish and fish products	2007	5	172	0
	2008	1	2	0
	2009	1	2	0
	2012	5	80	3
	2013	2	15	0
Total – Fish and fish products		14	271	3
Eggs and egg products	2007	1	4	0
	2009	2	19	3
	2011	2	15	0
	2012	1	3	3
	2013	2	19	0
Total – Eggs and egg products		8	60	6
Sweets and chocolate	2013	1	2	NR
	2014	1	8	0
Total – Sweets and chocolate		2	10	0
Vegetables and juices and other products thereof	2008	2	7	0
	2009	2	14	NR
	2010	2	4	0
	2011	4	189	0
	2012	1	4	0
	2013	6	64	0
	2014	2	238	15
Total – Vegetables and juices and other products thereof		19	520	15
Herbs and spices	2007	2	149	0
	2009	2	9	0
	2011	4	78	0
	2013	2	6	0
Total – Herbs and spices		10	242	0
Drinks, including bottled water	2013	1	7	0
Total – Drinks, including bottled water		1	7	0
Other foods	2007	31	241	23
	2008	3	306	2
	2009	13	269	53
	2010	2	22	0
	2011	8	81	6
	2012	2	12	0
	2013	8	90	2
	2014	11	219	22
Total – Other foods		78	1,240	108
Unknown	2007	28	303	3
	2008	5	128	0
	2009	2	19	NR
Total – Unknown		35	450	3
Total outbreaks		413	6,657	352

NR: not reported.

Table A.2: Reported outbreaks by implicated food vehicle where *Bacillus* other than *B. cereus* was implicated in reporting countries in accordance with Directive 2003/99/EC, from 2007 to 2014

Bacillus other than *B. cereus*	Food vehicle	Year	Total number of food-borne outbreaks	Human cases	Hospitalisations
Bacillus – *Bacillus* spp., unspecified	Other foods	2009	1	120	50
	Vegetables and juices and other products thereof	2012	1	NR	NR
	Cheese	2012	1	33	2
Total – *Bacillus* spp., unspecified			3	153	52
Bacillus – *B. subtilis*	Mixed food	2010	1	84	0
Total – *B. subtilis*			1	84	0
Total outbreaks			4	237	52

NR: not reported.

Appendix A – *Bacillus cereus* and *Bacillus* spp. food-borne outbreak data in the European Union Member States (MSs) and European non-MSs (2007–2014)

Table A.1: Reported strong-evidence food-borne outbreaks by implicated food vehicle where *Bacillus cereus* was implicated in reporting countries in accordance with Directive 2003/99/EC, from 2007 to 2014

Dal 2007 al 2014 gli Stati membri hanno segnalato **413** focolai basati su prove oggettive di origine alimentare collegata a *Bacillus cereus*, che hanno colpito **6657** persone, causando **352** ricoveri

Food vehicle	Year	Total number of food-borne outbreaks	Human cases	Hospitalisations
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	2009	1	20	NR
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	2011	1	3	3
	2012	1	2	0
	2013	1	10	0
Total – Cheese, milk and dairy products		11	65	3

Prodotti lattiero-caseari

episodi n. soggetti ricoveri

Nel 2005 in Italia, il *Ministero della Salute* riporta 17 notifiche relative alla presenza del microrganismo in prodotti alimentari

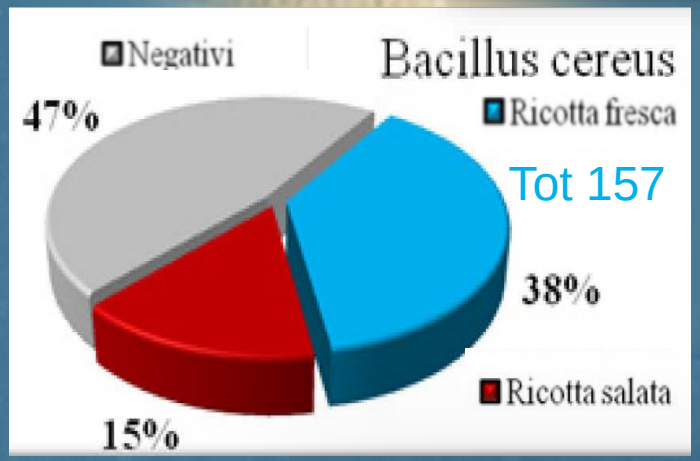
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

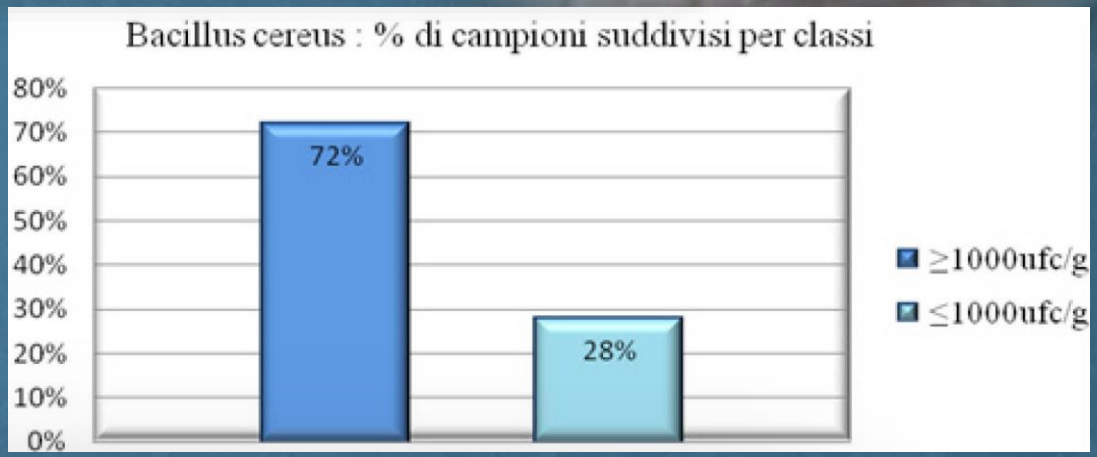
Italian Journal of Food Safety, Vol. 1 N. 5 Settembre 2012

PRESENZA DI *BACILLUS CEREUS*, *ESCHERICHIA COLI* E *ENTEROBACTERIACEAE* IN RICOTTA FRESCA E SALATA: CONTROLLI UFFICIALI NEL PERIODO 2009 – 2012.

Presence of Bacillus cereus, Escherichia coli and Enterobacteriaceae in fresh and salted Ricotta cheese: official controls in Sardinia during the period 2009 – 2012.

Fadda Antonio*, Delogu Alida, Mura Elia, Noli Alessia Caterina, Porqueddu Giuseppina, Rossi Maria Lucia, Terrosu Giovanni





efsa
European Food Safety Authority

Soglia di sicurezza: 1000 ufc/gr

Retrospective study on the hygienic quality of fresh ricotta cheeses produced in Sicily, Italy

Maria Luisa Scatassa,[1] Isabella Mancuso,[1] Sonia Sciortino,[1] Giusi Macaluso,[1] Marisa Palmeri,[1] Luigi Arcuri,[2] Massimo Todaro,[3] Cinzia Cardamone[1]

[1]Institute for Experimental Veterinary Medicine of Sicily "A. Mirri", Palermo; [2]Local Health Unit, Palermo; [3]Department of Agricultural, Food and Forest Sciences, University of Palermo, Italy

Table 1. Microbial loads of ricotta samples.

Microorganisms*	Number of samples	Positive samples	% positive samples
TMC	371	350	94.34
Rod LAB (37°C)	98	74	75.51
Coccus LAB (30°C)	98	82	83.67
Coccus LAB (44°C)	98	69	70.41
Enterococci	106	40	37.74
Enterobacteriaceae	371	78	21.02
E. coli	598	78	13.04
CPS	639	14	2.19
Yeasts and moulds	88	12	13.64
B. cereus	157	25	15.92
Pseudomonas	91	2	2.20
SRA	194	1	0.52
L. monocytogenes	1156	n.d.	n.d.
Salmonella spp.	998	n.d.	n.d.
Brucella spp.	721	n.d.	n.d.

*Units are log CFU/g. TMC, total mesophilic microorganisms; CPS, coagulase-positive staphylococci, SRA, Sulphite reducing anaerobe; n.d. not det

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[Italian Journal of Food Safety 2018; 7:6911]

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[Italian Journal of Food Safety 2018; 7:6911]

Food Control 79 (2017) 272–278

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/foodcont

Bacillus cereus in fresh ricotta: Comparison of growth and Haemolysin BL production after artificial contamination during production or post processing

Erica Tirloni [a, *], Emilia Ghelardi [b], Francesco Celandroni [b], Cristian Bernardi [a], Riccardo Casati [c], Per Sand Rosshaug [d], Simone Stella [a]

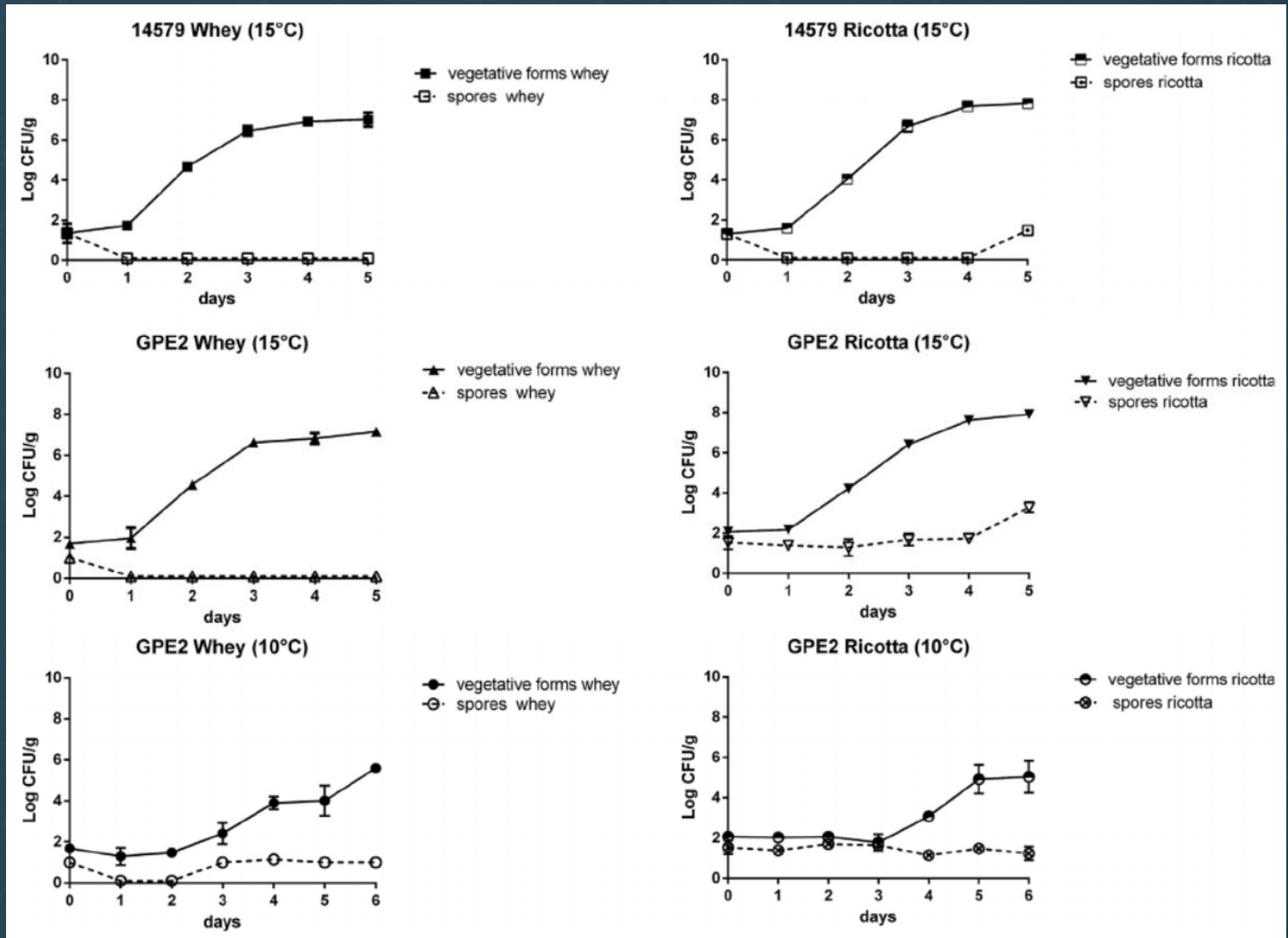


Fig. 1. Growth of *B. cereus* GPe2 and 14579 inoculated in the whey or in the final product (ricotta) and survival of their spores.

Toxin Detection Kits

BCET-RPLA TOXIN DETECTION KIT

Code: TD0950

A kit for the detection of Bacillus cereus enterotoxin (diarrhoeal type) in foods and culture filtrates by reversed passive latex agglutination.

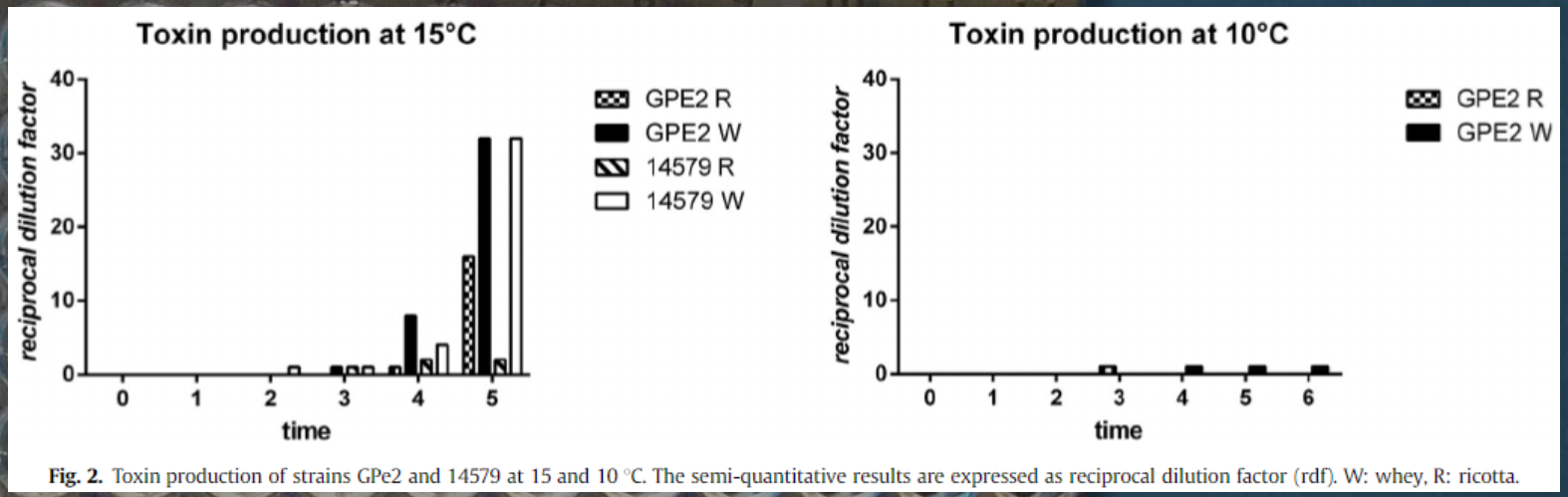


Fig. 2. Toxin production of strains GPe2 and 14579 at 15 and 10 °C. The semi-quantitative results are expressed as reciprocal dilution factor (rdf). W: whey, R: ricotta.

The fresh ricotta production process seems not to be completely safe, as

- spores survived (also if partially) to the thermal treatments
- bacteria multiplied in the product
- toxin is produced during bacterial growth (at 15°C)

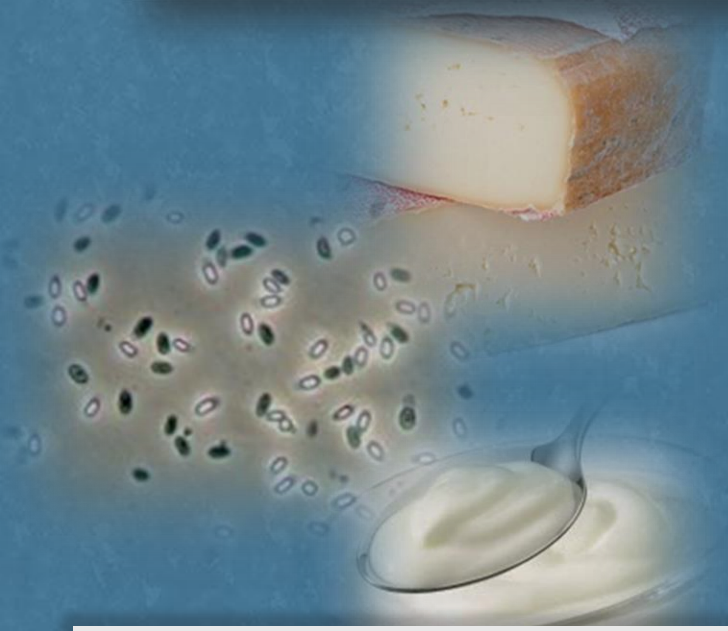
The contamination of the product after opening the pack should be regarded as a potential risk for the consumer

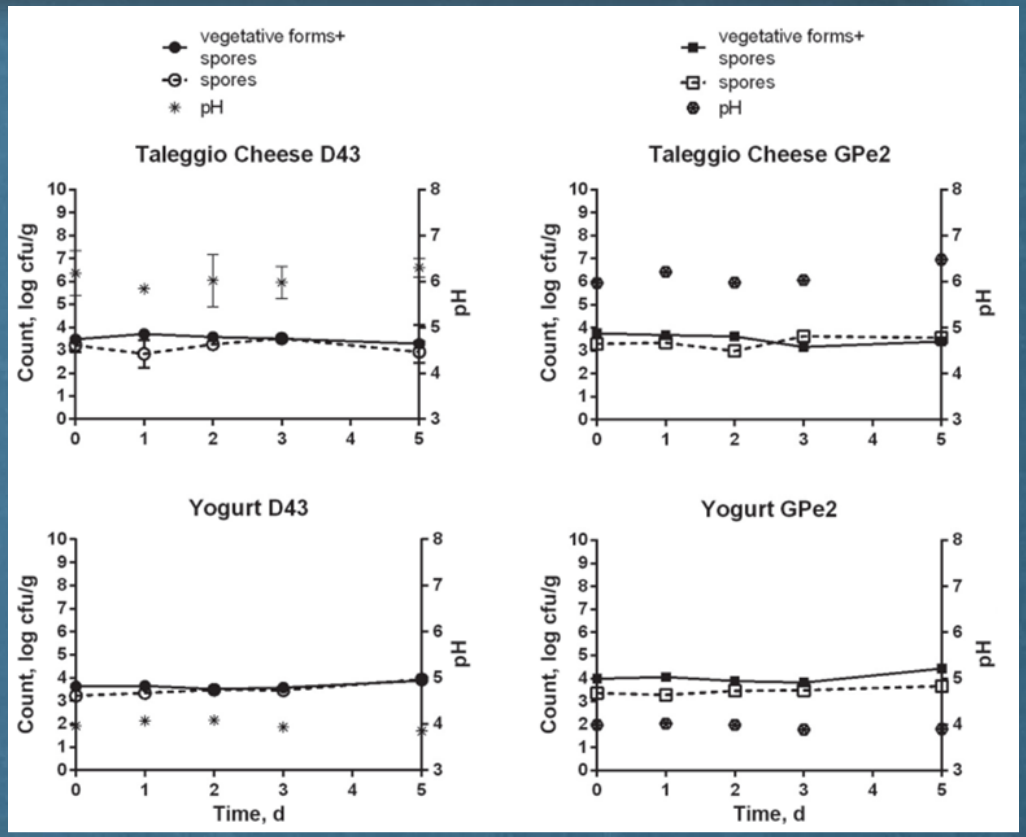
J. Dairy Sci. 100:1–9
https://doi.org/10.3168/jds.2017-12978

Journal of Dairy Science®
Official Journal of the American Dairy Science Association®

Effect of dairy product environment on the growth of *Bacillus cereus*

E. Tirloni,*[1] E. Ghelardi,† F. Celandroni,† C. Bernardi,* and S. Stella*
*Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Celoria 10, IT-20133, Milan, Italy
†Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via San Zeno 37, IT-56127, Pisa, Italy





Mean counts of *Bacillus cereus* GPe2 and D43 and their spores inoculated in Taleggio cheese and unflavored yogurt stored at 15°C for up to 5 d (sampling times at T0 d of inoculation, T1 after 24 h, T2 after 48 h, T3 after 72 h, and T5 after 120 h of storage). The pH values are also reported for each sampling time (refers to right axis). Error bars indicate SD.

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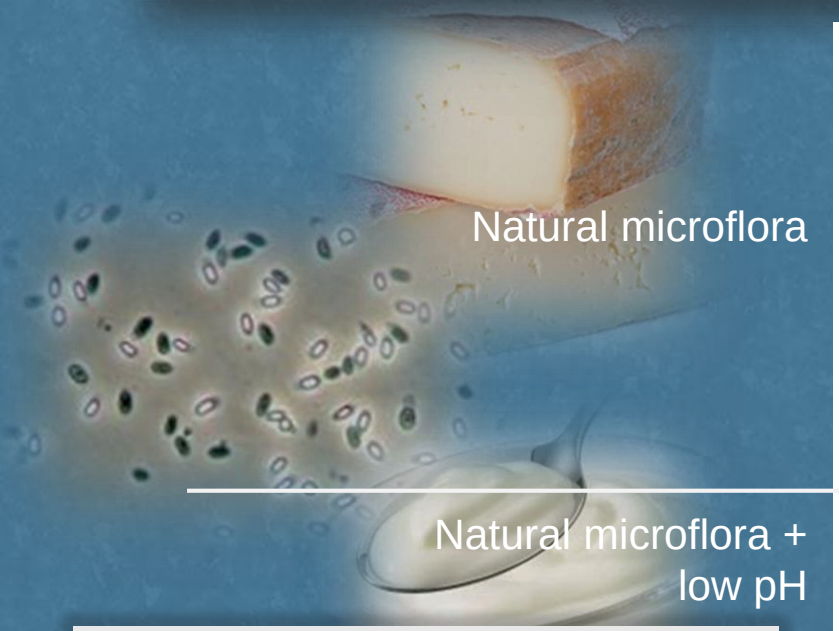
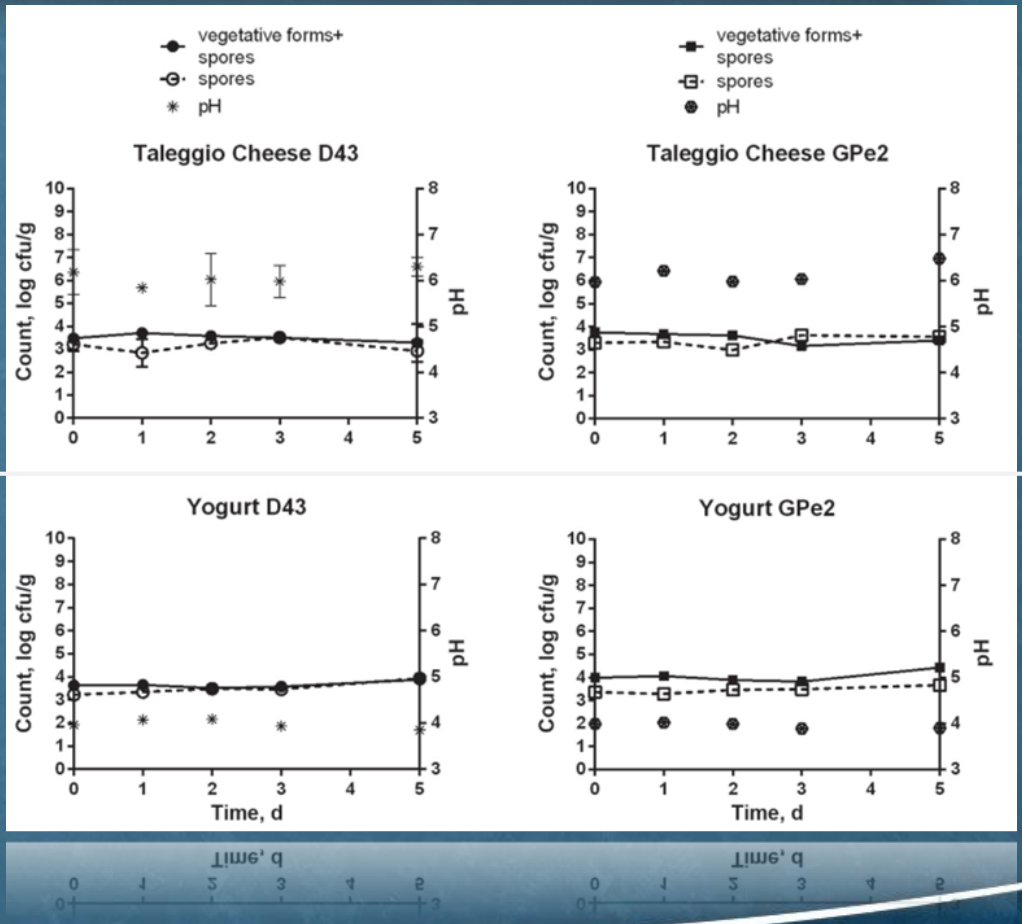
Effect of dairy product environment on the growth of *Bacillus cereus*

E. Tirloni,*[1] E. Ghelardi,† F. Celandroni,† C. Bernardi,* and S. Stella*
*Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Celoria 10, IT-20133, Milan, Italy
†Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via San Zeno 37, IT-56127, Pisa, Italy



Natural microflora

Natural microflora + low pH

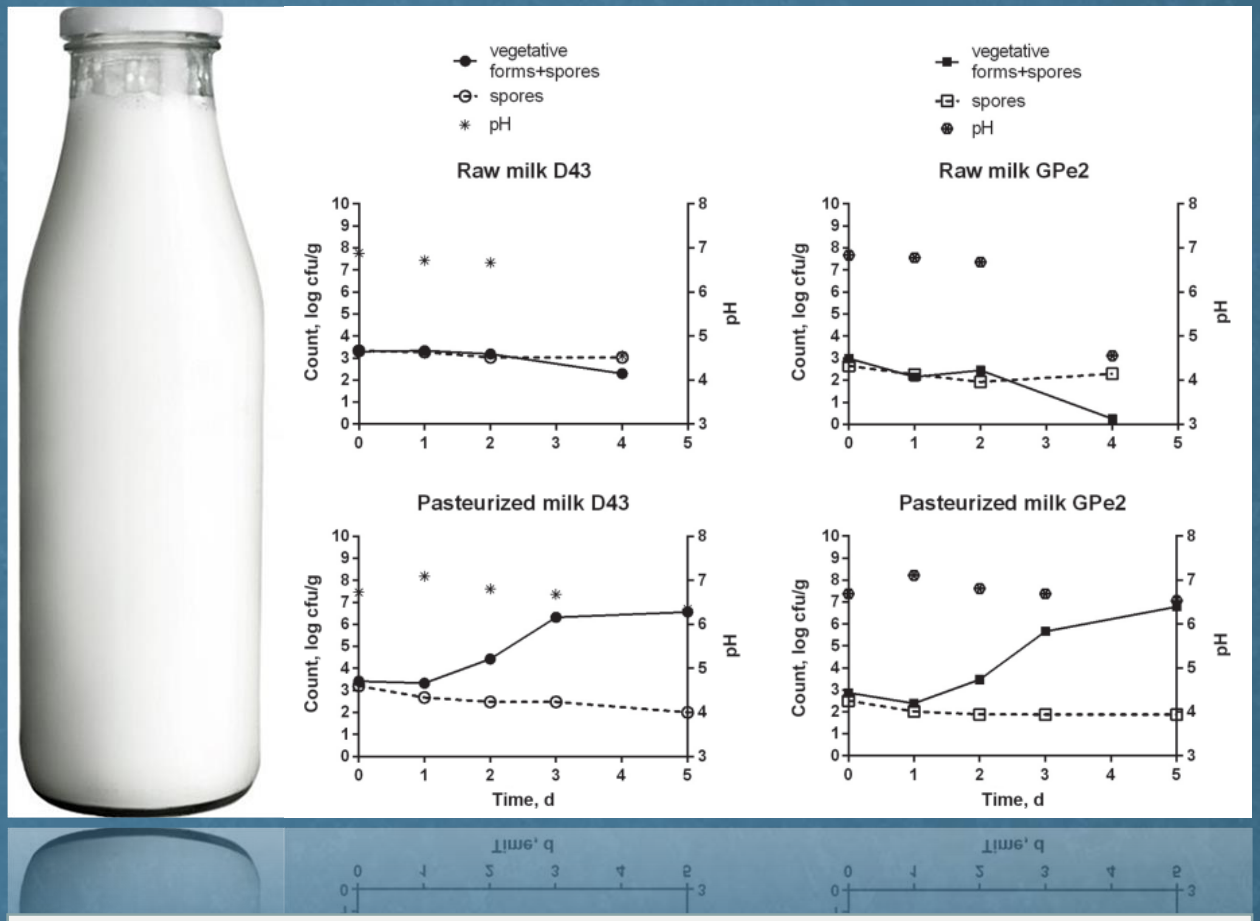


Mean counts of *Bacillus cereus* GPe2 and D43 and their spores inoculated in Taleggio cheese and unflavored yogurt stored at 15°C for up to 5 d (sampling times at T0 d of inoculation, T1 after 24 h, T2 after 48 h, T3 after 72 h, and T5 after 120 h of storage). The pH values are also reported for each sampling time (refers to right axis). Error bars indicate SD.

Table 1. Growth of *Bacillus cereus* GPe2 and D43 in nutrient broth at 2 different temperatures (15 and 37°C) recorded daily from 24 h postinoculation (T1) to 192 h (T8) as difference in optical density (OD)[1]

Strain and temperature	pH	T1	T2	T3	T4	T5	T6	T7	T8
GPe2 at 15°C									
	3.5	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—
	4.5	—	—	—	—	—	—	—	—
	5	—	—	—	—	—	—	—	—
	5.5	—	—	—	—	+	++	++	+++
	6	—	—	—	++	+++	+++	+++	+++
	6.5	—	—	+	++	++	++	++	++
	7	—	—	—	+++	+++	+++	+++	+++
	7.5	—	—	—	+++	+++	+++	++	++
GPe2 at 37°C									
	3.5	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—
	4.5	—	—	—	—	—	—	—	—
	5	—	—	++	+++	+++	+++	+++	+++
	5.5	+	+++	+++	+++	+++	+++	+++	+++
	6	++	+++	+++	+++	+++	+++	+++	+++
	6.5	++	+++	+++	+++	+++	+++	+++	+++
	7	+++	+++	+++	+++	+++	+++	+++	+++
	7.5	+++	+++	+++	+++	+++	+++	+++	+++
D43 at 15°C									
	3.5	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—
	4.5	—	—	—	—	—	—	—	—
	5	—	—	—	—	—	—	—	—
	5.5	—	—	—	—	—	—	—	—
	6	—	—	—	+	++	+	+	+
	6.5	—	—	—	+++	+++	++	++	+++
	7	—	—	—	+++	+++	++	++	+++
	7.5	—	—	—	+++	+++	++	++	++
D43 at 37°C									
	3.5	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—
	4.5	—	—	—	—	—	—	—	—
	5	—	+	+++	++	+++	+++	+++	+++
	5.5	+	+++	+++	+++	+++	+++	+++	+++
	6	+++	+++	+++	+++	+++	+++	+++	+++
	6.5	+++	+++	+++	+++	+++	+++	+++	+++
	7	+++	+++	+++	+++	+++	+++	+++	+++
	7.5	+++	+++	+++	+++	+++	+++	+++	+++

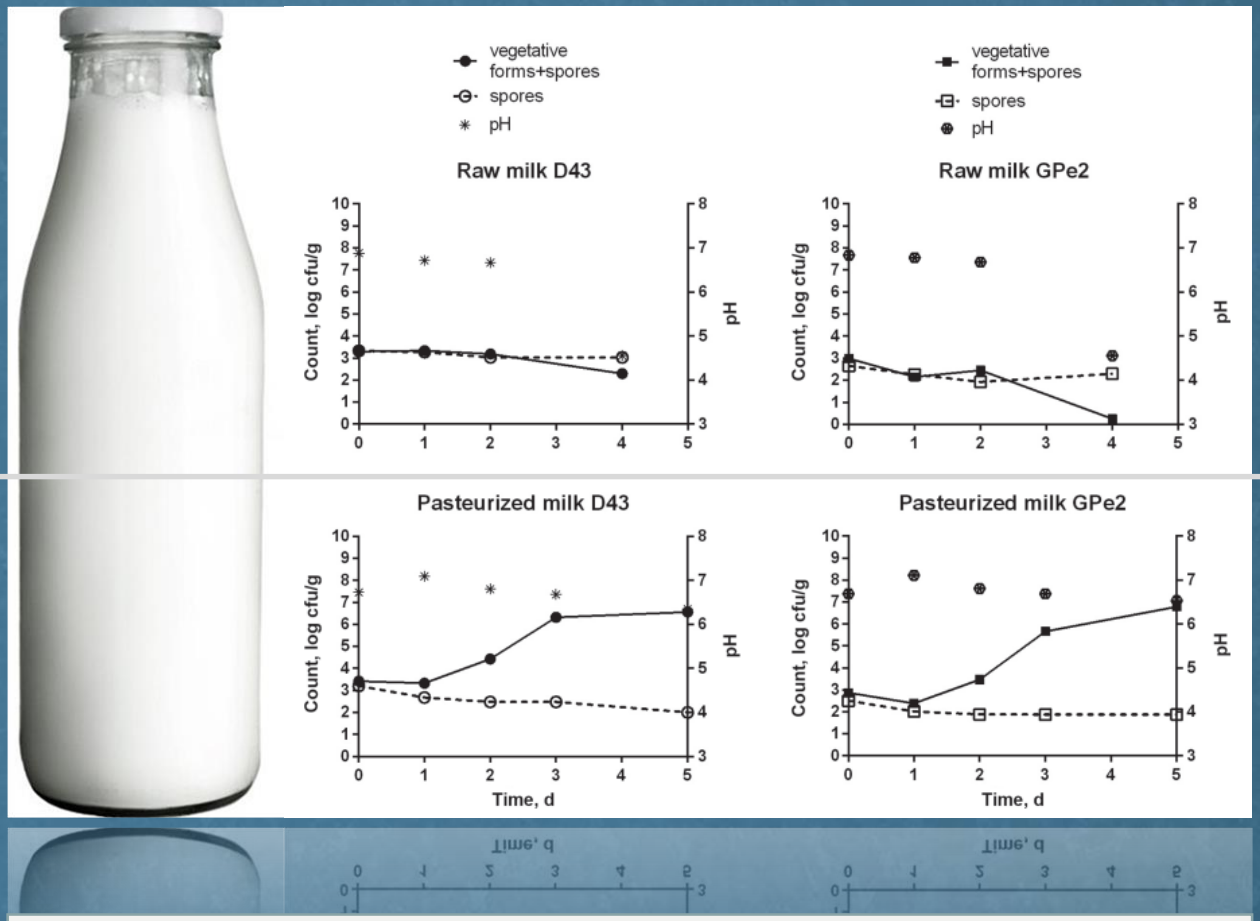
[1]+: increase <0.2 OD if compared with the equivalent blank sample. ++: increase between 0.2 and 0.5 OD if compared with the equivalent blank sample. +++: increase >0.5 OD if compared with the equivalent blank sample.



Mean counts of *Bacillus cereus* GPe2 and D43 and their spores inoculated in raw and pasteurized milk stored at 15°C for up to 4 d (sampling times at T0 d of inoculation, T1 after 24 h, and T4 after 96 h of storage). The pH values are also reported for each sampling time (refers to right axis)

Natural microflora

No microflora



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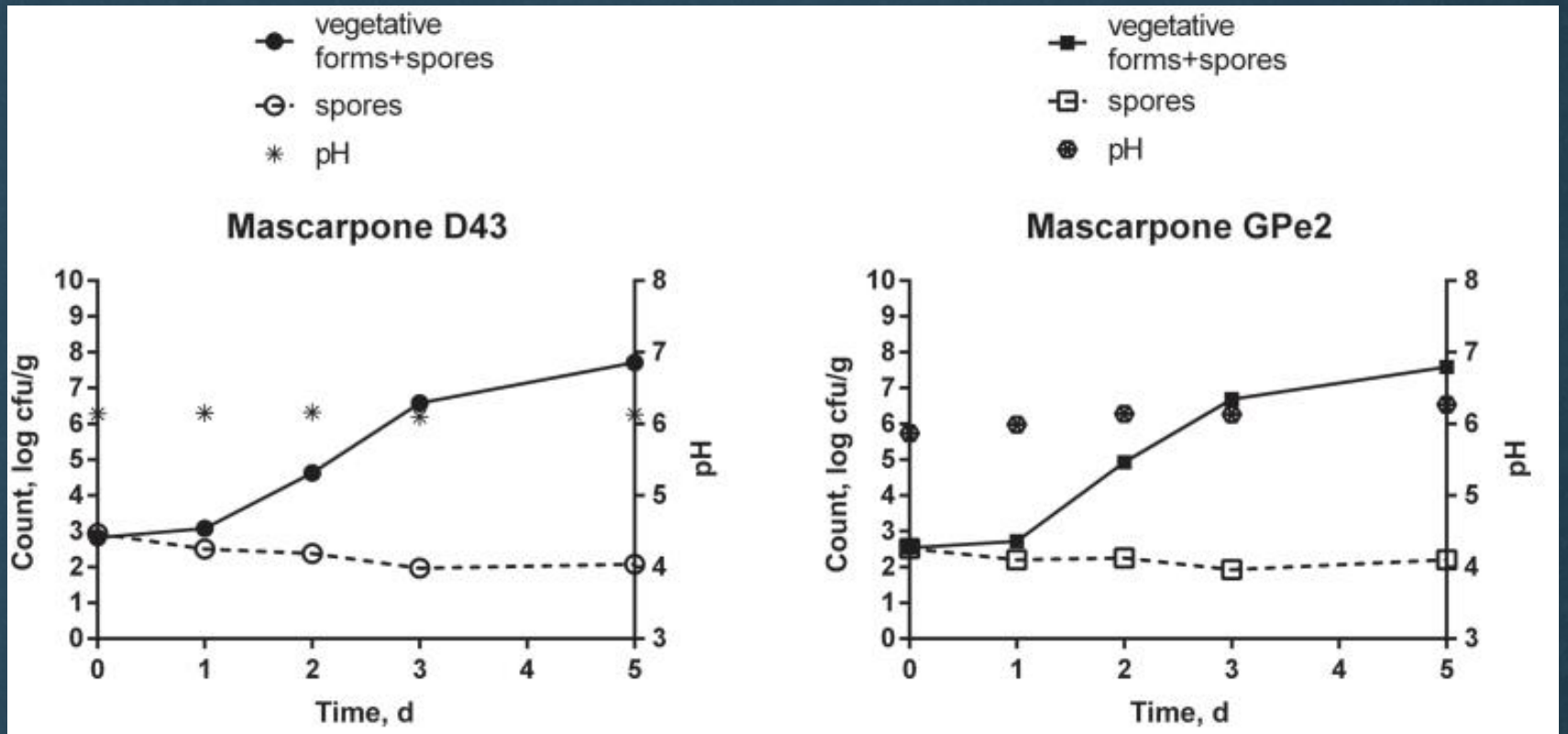
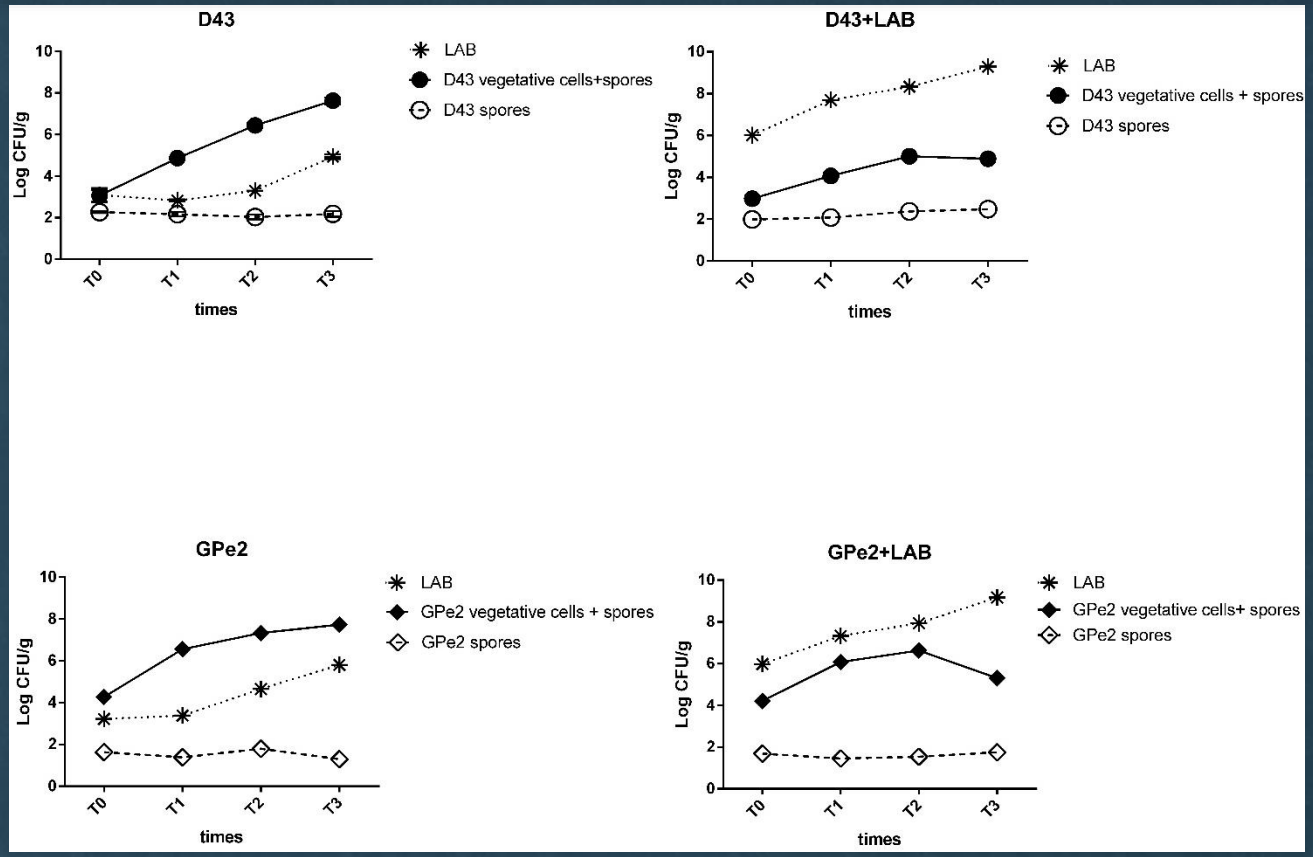


Figure 3. Mean counts of *Bacillus cereus* GPe2 and D43 and their spores inoculated in mascarpone cheese stored at 15°C for up to 5 d (sampling times at T0 d of inoculation, T1 after 24 h, T2 after 48 h, T3 after 72 h, and T5 after 120 h of storage). The pH values are also reported for each sampling time (refers to right axis).

Control of *Bacillus cereus* growth in fresh cheese by dairy microflora

E. Tirloni, C. Bernardi, E. Ghelardi, F. Celandroni, C. Andrighetto, N. Rota, S. Stella



Growth curves of *Bacillus cereus* GPe2 and D43 in fresh cheese with or without addition of LAB cultures

Halos produced by antagonistic activity of *Lactococcus* strains LAB 3, LAB 8 and LAB 1 against *Bacillus cereus* GPe2.

Conclusions

The production of spores, which are highly adhesive and can spread from natural *B. cereus* habitats to food production environments, accounts for the ability of *B. cereus* to contaminate any kind of food.

The high frequency of food contamination by *B. cereus* and the active production and secretion of HBL, Nhe and CytK enterotoxins, explain why this organism is responsible for food-poisoning related diseases.

However, further study are required to explain the mechanisms by which the toxins and enzymes *B. cereus* produces contribute to development and progression of diseases

The prevalence of *B. cereus* gastrointestinal infections is vague and most likely underestimated

B. cereus can be present in fresh and salted cheese

B. cereus growth in cheese is dependent of temperature, pH and microflora of the aliment

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